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High analgesic and anti-inflammatory in vivo activities of six new hybrids NSAIAs tetrahydropyran derivatives

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ABSTRACT

We present in this article syntheses of six new hybrids compounds (**4**–**9**) that were efficiently prepared in one or two steps (70–84.6%) from our previous prototype (±)-*cis*-4-chloro-6-(naphthalen-1-yl)-tetrahydro-2*H*-pyran-2-yl)methanol (**3**) and the NSAIAs: acetyl salicylic acid, indomethacin, ibuprofen, ketoprofen, naproxen and diclofenac. The acetic acid-induced writhing method is able to determine that all investigated new hybrids showed stronger antinociceptive properties (2- to 10-fold less ED_{50} values) than their precursors. The highest antinociceptive effect was observed for compound **9** showing more than 10-fold less ED_{50} values than diclofenac and ninefold less ED_{50} value than compound **2**. All compounds presented greater activity than the control group in the tail-flick test confirming the central antinociceptive effect. New hybrids did not alter the motor performance of mice by rota-rod performance and open-field tests. Investigated compounds **4**–**9** were not toxic after oral administration (LD_{50} >2000 mg/kg).

1. Introduction

Chronic pain is a leading cause of morbidity, reported to affect 19–50% of the European population.[1] Moreover, despite the large number of marketed analgesic and painkiller drugs, many sufferers are (or slowly become) insensitive to the currently available pharmacological approaches, and to date no adequate therapies are available for some common types of chronic pain, such as neuropathic pain or migraine.[2]

One of the significant developments in the medicinal chemistry over the last few years was the design and synthesis of hybrid compounds, molecules encompassing in a single scaffold two pharmacophores from known entities endowed with well established biological activities. Molecular hybridization approach is a powerful medicinal chemistry tool for designing ligands and prototypes promoting a beneficial effect to the treatment of multifactorial diseases.[3] One successful example of this strategy was the discovery of hybrid drug Benorylate (4-acetamidophenyl 2-acetoxybenzoate, **1**, Fig. 1) has been used as anti-inflammatory and antipyretic.[4] This hybrid drug was prepared by esterification of acetyl salicylic acid and paracetamol. It has a longer duration of action than aspirin[5] and only needs to be taken twice daily. Besides that, it has fewer side effects than aspirin, including gastric irritation and bleeding.

In the previous articles we described the first diastereoselective synthesis of (±)-*cis*-(6-ethyl-tetrahydropyran-2-yl) formic acid (**2a**),[6] and for the (−)-(*S*,*S*)-**2b** acid[7] (Fig. 1) both of them using Prins-cyclization reactions[8] as key-step on synthetic strategy to construct the tetrahydropyran skeletons. Compound **2a** presented important antinociceptive (analgesic and anti-inflammatory) properties. This compound was proposed as responsible for the bioactivity of the isolated *Vitex cymosa* sp. extract.[9] However, when spectroscopic data of synthetic **2a** were compared with the natural product we have found that lactone **2c**[10] was the actual natural product responsible for the analgesic activity of *V. cymosa* sp. (Fig. 1). Fortunately, this mistake[9] was very convenient for us, considering that unpublished **2a** and **2b** compounds showed higher antinociceptive activities than **2c**, and so compound **2a** and **2b** could be presented as a new promising nonsteroidal prototype to antinociceptive class of drugs. The antinociceptive activity of **2a** was evaluated in mice on acetic acid-induced abdominal writhing, on tail-flick test, on hotplate test, on formalin test, on reduction of spontaneous activity.[11] We described that the opioid receptor antagonist Naloxone totally reverted the effects of **2a** in all models. In fact, the pharmacological profile described for **2a** indicated that the substance can mediate antinociception at peripheral and central sites even when orally administered through activation of

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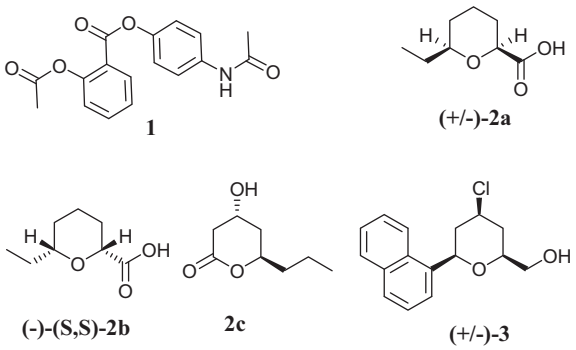


Figure 1. Benorylate (**1**) and the previous prototypes prepared by us (**2** and **3**).

opioid receptors.[12] Moreover, **2a** induced less tolerance, when compared to morphine.

In 2012 we reported the diastereoselective synthesis and high in vivo antinociceptive activity of the alcohol (±)-*cis*-2,4,6-**3** (Fig. 1), the most potent compound in a series of ten congener substances.[13] This congener series was proposed based on the bioisosterism strategy from **2a**[14] and lead to compound (±)-**3** being our prototype for discovery of more potent NSAIAs.

In our continuing search for bioactive substances[15–19] we present in this article the synthesis and in vivo antinociceptive/toxicological evaluations to tetrahydropyranyl derivatives esters **4**–**9** (Fig. 2). Compounds **4**–**9** were designed by molecular hybridization strategy from the analgesic alcohol **3** (moiety in blue, Fig. 2) with the non-steroidal anti-inflammatory agents/analgesics (NSAIAs), respectively: acetyl salicylic acid, indomethacin, ibuprofen, ketoprofen, naproxen and diclofenac potassium (moieties in black, Fig. 2).

2. Results and discussion

2.1. Chemistry

We began our experimental work on the diastereoselective synthesis of our prototype compound *cis*-(±)-**3** (Fig. 1) that was performed from Prins-cyclization reaction of 2-oxoethyl isobutyrate and 1-naphthaldehyde as described previously by us.[13] After that, the hybrid compound **4** was prepared from one-pot esterification of commercial salicylic acid chloride with alcohol (±)-**3** in presence of triethylamine as catalyst and dichloromethane as solvent (Scheme 1). Compounds **5**–**8** were prepared by esterifications of carboxylic acid chlorides which were prepared from carboxylic acids present in commercial drugs. Curiously, preparation of **9** was only efficiently performed in two steps (v and vi, Scheme 2) unlike the previous compounds. The optimized conditions for **5**–**9** preparations are shown in Scheme 2.

2.2. Biology

2.2.1. Effect of new hybrids on acetic acid-induced writhing

Intraperitoneal injection of acetic acid (1.2%) induced a total of 51 ± 8.1 writhes in a period of 30 min. The mice were pre-treated orally with new hybrids at doses of 1 mg/kg, 5 mg/kg and 10 mg/kg, control and vehicle (Table 1). With the purpose to better compare the molecular activities of compound **4**–**9** with commercial drugs and compound **3**, the ED_{50} values in Table 1 are described in μmol/kg.

The acetic acid-induced writhing method is able to determine antinociceptive effects of compounds and dose levels that might seem to be inactive in other methods.[21] In this test, all investigated new compounds showed stronger antinociceptive properties than their precursors (2- to 10-fold less ED_{50} values, Table 1). The most potent effect (ED_{50} values less than 5 μmol/kg p.o.) was produced by compounds **8** and **9**. The ED_{50} values of the investigated compounds at acetic acid-induced writhing method and their precursors are summarized in Table 1. The highest analgesic effect in acetic acid-induced writhing method was observed for compound **9**. It showed more than 10-fold less ED_{50} value than that of diclofenac and ninefold less ED_{50} value than that of compound **3**. It is worth noting in Table 1 that the hybrid compounds **8** and **9** are much more active than the corresponding molar mixtures 1:1 of precursor compounds (**3** + naproxen and **3** + diclofenac) which supports the success of our molecular hybridization strategy. The hybridization process is closely related to the strategy of obtaining a mutual prodrug, with the main difference being that the prodrug

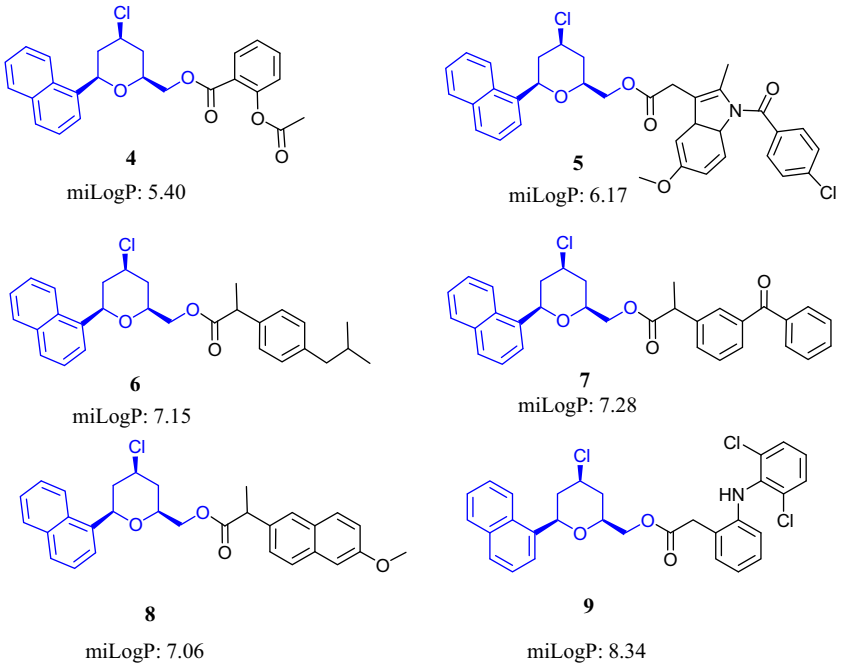
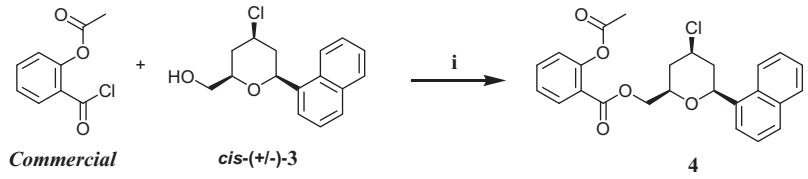
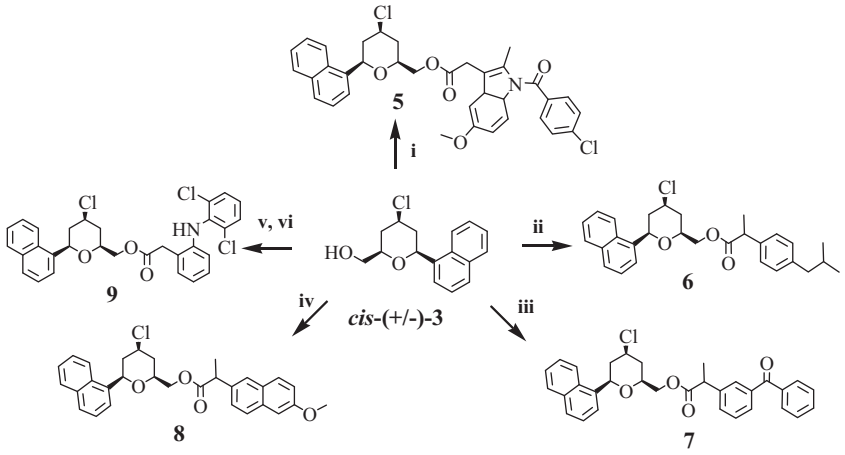


Figure 2. New synthesized and in vivo bioevaluated hybrids. Lipophilicity parameters (mi Log *P*) calculated by using the Molinspiration program.[20]



Scheme 1. Reagents and conditions: (i) salicylic acid chloride (1.2 mmol), CH_2Cl_2 (3.0 mL), TEA (0.4 mL), alcohol (±)-**3** (1.0 mmol), 0 °C (1 h), 24 h at rt, 92% yield.



Scheme 2. The optimized conditions for **5**–**9** preparations. Reagents and conditions: (i) indomethacin, oxalyl chloride, CH_2Cl_2, DMF (cat.), 0 °C; (±)-**3**, CH_2Cl_2, TEA, 7 days, rt, 70%; (ii) ibuprofen, $SOCl_2$, CH_2Cl_2, rt; (±)-**3**, CH_2Cl_2, TEA, 7 days, 80 °C, 70%; (iii) ketoprofen, $SOCl_2$, CH_2Cl_2, rt; (±)-**3**, CH_2Cl_2, TEA, 7 days, 80 °C, 72%; (iv) naproxen, $SOCl_2$, CH_2Cl_2, rt; (±)-**3**, CH_2Cl_2, TEA, 7 days, 80 °C, 74%; (v) (±)-**3**, TsCl, CH_2Cl_2, TEA, 12 h, rt, 94%; (vi) diclofenac potassium, K_2CO_3, DMF, MW (100 W), 100 °C, 90 min, 90%.

Table 1
Calculated ED_{50} values from the acetic acid-induced abdominal writhes in mice

Compounds	ED_{50} values (μmol/kg)
4	20.39
Acetyl salicylic acid	44.16
5	13.77
Indomethacin	72.62
6	10.30
Ibuprofen	60.26
7	14.03
Ketoprofen	46.60
8	4.60
Naproxen	21.76
9	3.17
Diclofenac	32.48
3	27.97
1:1 Molar mixture (naproxen + **3**)	10.33
1:1 Molar mixture (SCHEME + **3**)	9.65

action is dependent on its in vivo cleavage while hybrid compounds can also act 'per se' at their specific receptors or targets. So far we do not know the exact molecular target of our new drugs **4**–**9**. We previously described that compound (±)-**2a** (Fig. 1) acts at opioid receptors.[11] Even so, we cannot say yet the precise target of **4**–**9**. With the purpose of discovering the molecular receptors for compounds **4**–**9**, additional pharmacological studies (similar to those previously reported by us)[11,12] are now under investigation.

2.2.2. Effect of new hybrids derivatives in the tail-flick test

The tail-flick test was used to assess the central activity of the compounds, since this test is predominantly a spinal reflex, and is considered to be selective for centrally acting analgesic substances, whereas peripherally acting analgesics are known to be inactive against thermal stimuli.[22,23] Figure 3A–D shows that all the compounds presented a result significantly greater than the control group in the tail-flick test throughout the experiment, confirming the central antinociceptive effect of these compounds. In Figure 3A and C, graphs represent time–effect curve. In Figure 3B and D, graphs represent the area under the curve (AUC) calculated for each time–effect curve. The dose of compounds was 10 mg/kg (p.o.). The results are presented as percentage increase over the baseline or area under the curve (AUC); $n = 6$ per group. Statistical significance was calculated by the analysis of variance followed by Bonferroni's test. $^*P < 0.05$ relative to the control group. Where no error bars are shown, it is because they are smaller than the symbol.

2.2.3. Effect of new hybrids in the rota-rod performance and open field tests

The rota-rod performance (forced motor activity) and open-field (spontaneous motor activity) tests were used to exclude the possibility that the antinociceptive action of new hybrids could be related to nonspecific disturbances in the locomotors activity of the animals. We observed that at dose that has antinociceptive action (10 mg/kg, p.o.), new hybrids did not alter the motor performance of mice in both tests (Fig. 4).

2.2.4. Toxicological evaluation in vivo of new hybrids

All new hybrids described in this paper were evaluated for their acute toxicity in mice. Symptoms of intoxications were not observed in animals (disorientation, hyperactivity, piloerection and hyperventilation). Investigated compounds were not toxic in mice after oral administration (LD_{50} >2000 mg/kg).

3. Conclusion

The art of discovering new bioactive molecules that can be prepared at low cost, efficiently and compatible for large-scale preparation is the greatest objective in Medicinal Chemistry. Thus, we believe that a simple and efficient synthetic methodology to obtain a new drug will increase the importance of the process. In this

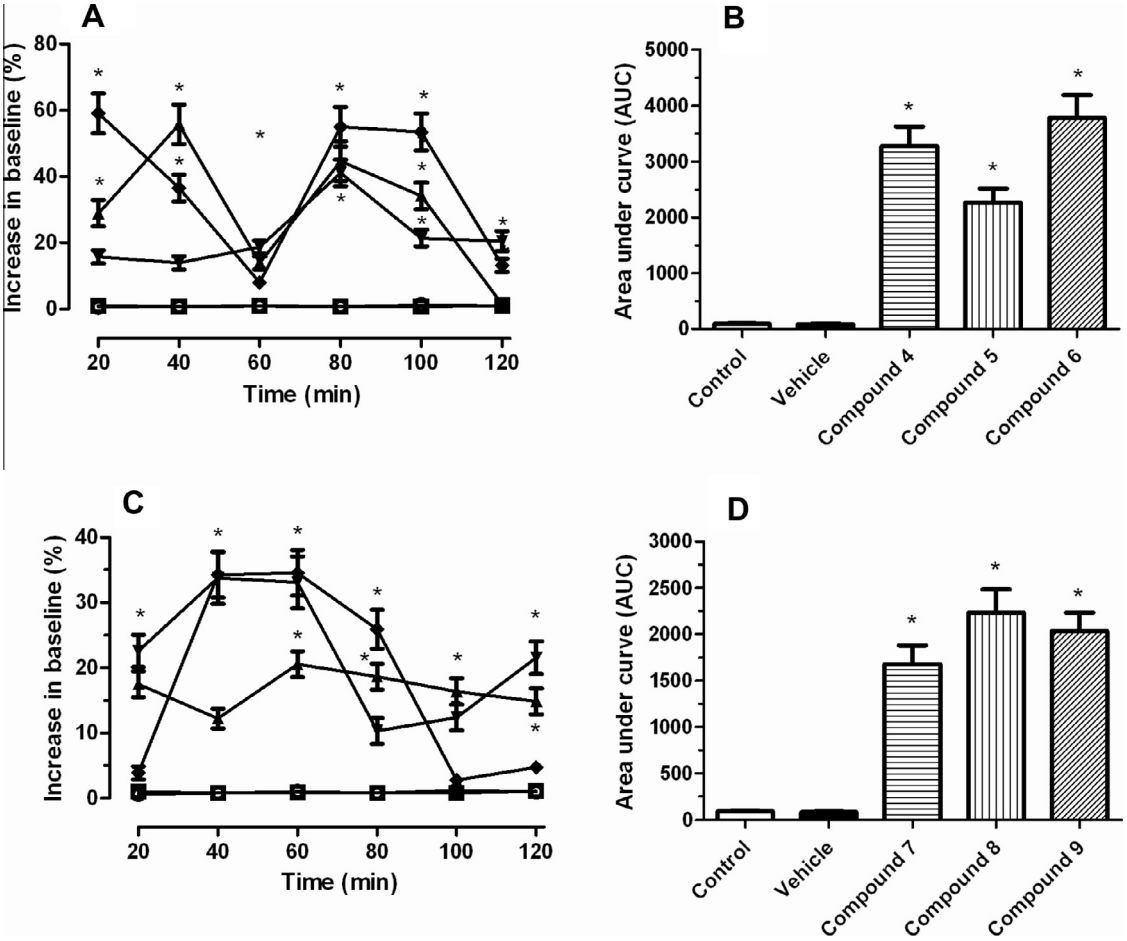


Figure 3. Effects of new hybrids in the tail-flick test. In (A), (–◯–) control, (–□–) vehicle, (–▲–) compound **4**, (–▼–) compound **5**, (–◆–) compound **6**. In (C), (–◯–) control, (–□–) vehicle, (–▲–) compound **7**, (–▼–) compound **8** and (–◆–) compound **9**. Statistical significance was calculated by the analysis of variance followed by Bonferroni's test. $^{*}P$ <0.05 relative to the control group.

focus, we presented here very simple and efficient syntheses (esterifications) and antinociceptive evaluation to six new hybrid compounds **4**–**9**. In vivo evaluations of acetic acid-induced writhing method, the tail-flick test, the rota-rod performance (forced motor activity) and open-field (spontaneous motor activity) tests demonstrated that new compound present very stronger antinociceptive properties than their precursors and did not alter the motor performance of mice. Compound **9** is the most active (ED_{50} = 3.17 μmol/kg) and selective compound, showed more than 10-fold less ED_{50} value than diclofenac and ninefold less ED_{50} value that of prototype **3**, which supports the success of our molecular strategy. The best of all was that compound **9** could be easily prepared by a simple esterification in high yields (two steps, 84% yield). It is important to highlight that compounds **4**–**9** are not toxic after oral administrations which suggest the high potentiality of these drugs for subsequent studies as new NSAIAs. Finally, we can note that we presented up to now, racemic syntheses to **4**–**9**. Enantioselective approaches for these compounds are now under investigation.[7]

4. Experimental procedures

4.1. Chemistry

4.1.1. General methods

All commercially available reagents and solvent were obtained from commercial providers and used without further purification. Microwave heating reactions were performed in a CEM Discover benchmate using the 10 mL Pyrex pressure vial for closed vessel reactions, under 100 W automatically to reach and maintain the set temperature, specified in each case, monitored by built-in infrared sensor and medium stirring speed using cylindrical stir bars (5 × 2 mm), default ramp time of 3 min. Reactions were monitored by TLC using Silica gel 60 UV_{254} Macherey Nagel pre-coated silica gel plates; detection was by means of a UV lamp and revelation to vanillin. Flash column chromatography was performed on 230–400 mesh silica gel. Organic layers were dried over anhydrous $MgSO_4$ or Na_2SO_4 prior to evaporation on a rotary evaporator. ^{1}H NMR and ^{13}C NMR spectra were recorded using Varian Mercury Spectra AC 20 spectrometer (400 MHz and 200 MHz for ^{1}H, 100 MHz and 50 MHz for ^{13}C) in $CDCl_3$. Chemical shifts were reported relative to internal tetramethylsilane (δ 0.00 ppm) for ^{1}H, and $CDCl_3$ (δ 77.0 ppm) for ^{13}C. FTIR spectra were recorded on a Shimadzu spectrophotometer model IRPrestige-21 in KBr pellets. MS data were measured with a Shimadzu GCMS e QP2010 mass spectrometer. The elemental analyses of unpublished compounds were performed in an analyser organic elementar CHNS-O, FLASH 2000 of Thermo Scientific.

4.1.2. Synthesis of *cis*-(±)-4-chloro-6-(naphthalen-1-yl)-tetrahydro-2*H*-pyran-2-yl)methyl 2-acetoxybenzoate (4)

The reaction was performed by stirring a solution of alcohol **3** (1.0 mmol) in CH_2Cl_2 (3.0 mL), triethylamine (0.4 mL) and the

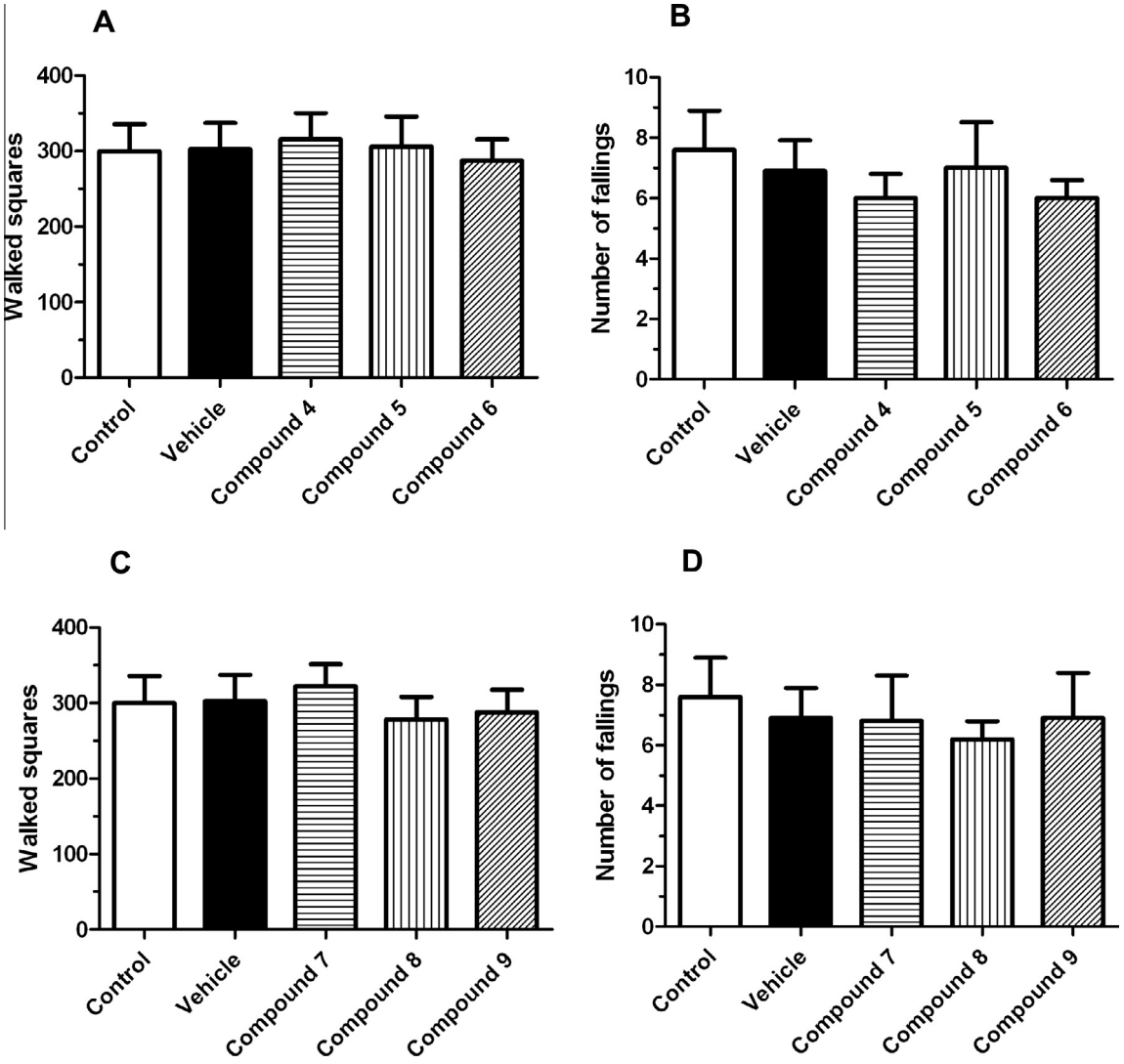


Figure 4. Effects of new hybrids in the rota-rod performance e and open-field tests. In (A) and (C), graphs represent the number of walked squares by mice In (B) and (D), graphs represent number of fallings of mice. The dose of compounds was 10 mg/kg (p.o.); $n = 6$ per group. Statistical significance was calculated by the analysis of variance followed by Bonferroni's test. $^{*}P < 0.05$ relative to the control group.

mixture were stirred at 0 °C. Additional 1.2 mmol of 2-(chlorocarbonyl) phenyl acetate (acetyl salicylic acid chloride) was charged to the reactions mixture. The reaction was continued for 24 h and mixture was allowed to room temperature. After the end of reaction (verified by chromatography using hexane:ethyl acetate (7:3) as solvent), the resulting mixture was extracted with CH_2Cl_2 (3×20 mL). Pure product was obtained from the crude reaction by column chromatography through silica gel, using AcOEt:hexane as solvent at a ratio of 3:7. The solvent mixture was concentrated under reduced pressure to afford **4** in 92% yield; IR (KBr, cm^{-1}): 914 and 786 (C–H aromatic), 1369–1296 (C–O), 1604 and 1446 (C=C aromatic), 1724 and 1766 (C=O ester), 2924 (C–H sp^3); ^1H NMR ($CDCl_3$, 400 MHz) δ: 1.86 (m, 1H), 2.11 (m, 2H), 2.25 (s, 3H), 2.37 (m, 1H), 2.61 (m, 1H), 4.06 (m, 1H), 4.32 (m, 2H), 4.48 (dd, 1H, $J = 16$ Hz), 5.12 (d, 1H, $J = 10$ Hz), 7.57 (m, 10H); ^{13}C NMR (101 MHz; $CDCl_3$) δ: 20.96, 38.49, 42.50, 55.04, 66.86, 75.11, 75.78, 122.80, 123.05, 123.32, 123.76, 125.38, 125.51, 125.96, 126.14, 128.54, 128.86, 130.31, 131.76, 133.69, 134.04, 135.88, 150.73, 164.12, 169.72. Anal. Calcd for $C_{25}H_{23}ClO_5$, C, 68.41; H, 5.28. Found: C, 68.37; H, 5.31.

4.1.3. Synthesis of *cis*-(±)-4-chloro-6-(naphthalen-1-yl)-tetrahydro-2*H*-pyran-2-yl)methyl 2-(1-(4-chlorobenzoyl)-5-methoxy-2-methyl-3a,7a-dihydro-1*H* indol-3-yl) acetate (5)

The reaction was performed by stirring a solution of indomethacin (5.6 mmol) in CH_2Cl_2 (10.0 mL) and 6.7 mmol of oxalyl chloride. The mixture was stirred at room temperature for 12 h. After the end of reaction (verified by chromatography using hexane:ethyl acetate (7:3) as solvent), the resulting mixture was extracted with CH_2Cl_2 (3×20 mL). The organic phase was dried with anhydrous sodium sulfate and concentrated under reduced pressure to afford indomethacin chloride in 95% yield. 1 mmol of indomethacin chloride and 1 mmol of alcohol (±)-**3** were stirred in 10 mL of THF and 0.5 mL of TEA for 7 days. After the end of reaction (verified by chromatography), the resulting mixture was extracted with CH_2Cl_2 (3×20 mL). Pure product was obtained from the crude reaction by column chromatography through silica gel, using AcOEt:hexane as solvent at a ratio of 3:7. The solvent mixture was concentrated under reduced pressure to afford **5** in 70% yield; IR (KBr, cm^{-1}): 798 and 779 (C–H of aromatic); 1354–1319 (C–O); 1597 and 1450 (C=C of

aromatic); 1735 (C=O of ester); 1681 (C=O of amide); 2924 (C–H sp^3); 3429 (N–H). ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 1.25 (t, 1H); 1.81 (sxt, 2H, J = 12 Hz); 2.12 (m, 2H); 2.36 (s, 3H); 2.55 (dd, 1H, J = 4 Hz); 3.68 (t, 2H, J = 4 Hz); 3.72 (m, 3H); 3.86 (m, 1H); 4.06 (m, 1H); 4.24 (m, 2H, J = 4 Hz); 5.09 (m, 1H); 6.79 (m, 2H); 7.66 (m, 12H). ^{13}C NMR (101 MHz; $CDCl_3$) δ: 13.37; 30.18; 38.24; 42.77; 54.97; 55.52; 66.59; 74.92; 75.64; 101.23; 111.53; 112.31; 114.92; 122.74; 125.50; 128.94; 128.99; 130.49; 131.06; 133.64; 133.73; 136.01; 155.94; 168.17; 170.59. Anal. Calcd for $C_{35}H_{33}Cl_2NO_5$, C, 68.18; H, 5.07; N, 2.27. Found: C, 68.19; H, 5.08; N, 2.22.

4.1.4. Synthesis of *cis*-(±)-4-chloro-6-(naphthalen-1-yl)-tetrahydro-2*H*-pyran-2-yl)methyl 2-(4-isobutylphenyl) propanoate (6)

The reaction was performed by stirring a solution of ibuprofen (9.7 mmol) in $SOCl_2$ (5.0 mL) and the mixture was stirred at room temperature at 80 °C for 3 h. After the end of reaction (verified by chromatography), the resulting mixture evaporated obtained 95% of ibuprofen chloride. 1 mmol of ibuprofen chloride and 1 mmol of alcohol (±)-**3** were stirred in 10 mL of THF and 0.5 mL of TEA at 80 °C for 7 days. After the end of reaction (verified by chromatography using hexane:ethyl acetate (7:3) as solvent), the resulting mixture was extracted with CH_2Cl_2 (3 × 20 mL). Pure product was obtained from the crude reaction by column chromatography through silica gel, using AcOEt:hexane as solvent at a ratio of 3:7. The solvent mixture was concentrated under reduced pressure to afford **6** in 70% yield; IR (KBr, cm^{-1}): 779 and 736 (C–H aromatic); 1377–1330 (C–O); 1512 and 1454 (C=C aromatic); 1732 (C=O of ester); 2954 (C–H sp^3). ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 0.86 (dd, 6H, J = 4 Hz); 1.51 (dd, 3H); 1.92 (m, 5H); 2.38 (dd, 2H, J = 4 Hz); 2.52 (m, 1H); 3.81 (m, 2H); 4.26 (m, 3H); 5.05 (d, 1H, J = 12 Hz); 7.01 (m, 2H); 7.22 (dd, 2H, J = 4 Hz); 7.53 (m, 3H); 7.89 (m, 3H). ^{13}C NMR (101 MHz; $CDCl_3$) δ: 18.31; 22.40; 30.11; 38.32; 42.87; 55.11; 66.18; 66.33; 75.04; 75.70; 122.88; 123.33; 125.54; 126.14; 127.18; 128.40; 128.96; 129.29; 130.13; 133.72; 136.17; 136.24; 137.42; 140.60, 174.51. Anal. Calcd for $C_{29}H_{33}ClO_3$, C, 74.90; H, 7.62. Found: C, 74.86; H, 7.57.

4.1.5. Synthesis of *cis*-(±)-4-chloro-6-(naphthalen-1-yl)-tetrahydro-2*H*-pyran-2-yl)methyl 2-(3-benzoylphenyl) propanoate (7)

The reaction was performed by stirring a solution of ketoprofen (2.0 g) in $SOCl_2$ (5.0 mL) and the mixture was stirred at room temperature at 80 °C for 3 h. After the end of reaction (verified by chromatography), the resulting mixture on evaporation afforded ketoprofen chloride in 95% yield. 1 mmol of ketoprofen chloride and 1 mmol of alcohol (±)-**3** were stirred in 10 mL of THF and 0.5 mL of TEA at 80 °C for 7 days. After the end of reaction (verified by chromatography using hexane:ethyl acetate (7:3) as solvent), the resulting mixture was extracted with CH_2Cl_2 (3 × 20 mL). Pure product was obtained from the crude reaction by column chromatography through silica gel, using AcOEt:hexane as solvent at a ratio of 3:7. The solvent mixture was concentrated under reduced pressure to afford **7** in 72% yield; IR (KBr, cm^{-1}): 786 and 721 (C–H aromatics); 1315–1172 (C–O); 1593 and 1450 (C=C aromatics); 1658 (C=O) 1735 (C=O ester); 2958 (C–H sp^3). ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 2.10 (d, 1H, J = 6 Hz); 2.48 (m, 1H); 2.73 (m, 3H); 2.91 (m, 2H); 3.11 (m, 1H); 3.39 (m, 1H); 3.74 (m, 1H); 4.66 (m, 1H); 5.01 (m, 2H); 5.31 (m, 1H); 5.47 (m, 2H); 8.73 (m, 16H). ^{13}C NMR (101 MHz; $CDCl_3$) δ: 19.74; 27.37; 30.37; 39.75; 44.24; 46.67; 56.41; 65.48; 67.88; 76.32; 124.23; 124.60; 126.93; 127.52; 129.66; 129.81; 130.33; 130.52; 131.43; 132.93; 133.86; 135.08; 137.49; 138.81; 139.19; 142.03; 142.20; 175.27; 197.82. Anal. Calcd for $C_{32}H_{29}ClO_4$: C, 74.92; H, 5.70. Found: C, 74.85; H, 5.62.

4.1.6. Synthesis of *cis*-(±)-4-chloro-6-(naphthalen-1-yl)-tetrahydro-2*H*-pyran-2-yl)methyl 2-(6-methoxynaphthalen-2-yl)propanoate (8)

The reaction was performed by stirring a solution of naproxen (2.0 g) in $SOCl_2$ (5.0 mL) and the mixture was stirred at room temperature at 80 °C for 3 h. After the end of reaction (verified by chromatography using hexane:ethyl acetate (7:3) as solvent), the resulting mixture on evaporation afforded naproxen chloride in 95% yield. 1 mmol of naproxen chloride and 1 mmol of alcohol (±)-**3** were stirred in 10 mL of THF and 0.5 mL of TEA at 80 °C for 7 days. After the end of reaction (verified by chromatography), the resulting mixture was extracted with CH_2Cl_2 (3 × 20 mL). Pure product was obtained from the crude reaction by column chromatography through silica gel, using AcOEt:hexane as solvent in a ratio of 3:7. The solvent mixture was concentrated under reduced pressure to afford **8** in 74% yield; IR (KBr, cm^{-1}): 825 and 800 (C–H aromatics); 1327–1178 (C–O); 1600 and 1500 (C=C aromatics); 1730 (C=O ester); 2970 (C–H sp^3). ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 0.84 (t, 2H); 1.24 (m, 1H); 1.59 (m, 5H); 1.71 (m, 2H); 3.44 (m, 1H); 3.87 (m, 1H); 4.01 (s, 3H); 4.04 (m, 1H); 4.10 (m, 2H); 7.28 (m, 2H); 7.52 (m, 4H); 7.70 (m, 5H); 8.17 (d, 2H). ^{13}C NMR (101 MHz; $CDCl_3$) δ: 10.29; 18.32; 18.43; 21.91; 25.93; 28.97; 44.41; 45.30; 56.97; 63.98; 66.44; 113.88; 113.94; 116.73; 123.88; 123.97; 126.12; 126.13; 127.39; 127.49; 127.81; 129.45; 129.50; 130.99; 131.03; 136.41; 136.63; 152.75; 174.50. Anal. Calcd for $C_{32}H_{29}ClO_4$: C, 74.92; H, 5.70. Found: C, 74.85; H, 5.62.

4.1.7. 4.1.7.Synthesis of *cis*-(±)-4-chloro-6-(naphthalen-1-yl)-tetrahydro-2*H*-pyran-2-yl)methyl 2-(2-(2,6-dichlorophenylamino) phenyl)acetate (9)

The reaction was performed by stirring a solution of alcohol **3** (0.53 mmol) in CH_2Cl_2 (2.0 mL), triethylamine (0.2 mL), 4-toluenesulfonyl chloride (1.3 mmol) and the mixture was stirred at 0 °C for 30 min. and 12 h at room temperature. After the end of reaction (verified by chromatography using hexane:ethyl acetate (7:3) as solvent), the resulting mixture was extracted with CH_2Cl_2 (3 × 20 mL). The organic phase was dried with anhydrous sodium sulfate and concentrated under reduced pressure where tosylate derivative of alcohol **3** was obtained a 95% yield. After, 0.1 mmol of tosyl derivative of alcohol **3** was stirred with 0.15 mmol of diclofenac Potassium, 22 mg of K_2CO_3 in 1 mL of DMF under microwave irradiation (100 W) at 100 °C for 90 min. Pure product was obtained from the crude reaction by column chromatography through silica gel, using AcOEt:hexane as solvent in a ratio of 3:7. The solvent mixture was concentrated under reduced pressure to afford **9** in 90%; IR (KBr, cm^{-1}): 777 and 742 (C–H aromatics); 1303 (C–O); 1577 and 1502 (C=C aromatics); 1691 (C=O ester); 2918 (C–H sp^3); 3348 (N–H). ^{1}H NMR ($CDCl_3$, 400 MHz) δ: 1.26 (t, 1H, J = 8 Hz); 1.81 (dd, 1H, J = 12 Hz); 2.09 (q, 2H, J = 12 Hz); 2.23 (m, 1H); 2.58 (t, 1H, J = 4 Hz); 2.61 (t, 4H, J = 4 Hz); 3.76 (m, 4H); 4.33 (m, 1H); 5.14 (d, 1H); 7.23 (m, 2H); 7.51 (m, 3H); 7.62 (d, 1H, J = 8 Hz); 7.85 (m, 2H); 8.00 (d, 1H, J = 8 Hz). ^{13}C NMR (101 MHz; $CDCl_3$) δ: 38.02; 38.15; 42.76; 55.39; 65.66; 75.70; 77.91; 118.24; 122.04; 122.89; 123.26; 123.90; 123.98; 125.37; 125.64; 126.29; 128.05; 128.60; 128.82; 128.96; 129.38; 130.32; 130.90; 133.73; 136.09; 142.60; 175.93. Anal. Calcd for $C_{30}H_{26}Cl_3NO_3$: C, 64.94; H, 4.72; N, 2.52. Found: C, 65.07; H, 4.71; N, 2.51.

4.2. Pharmacology

4.2.1. Animals

The experiments were carried out on male Albino–Swiss mice (body weight 20–24 g). The animals were housed in wire mesh cages in a room temperature and exposed to a 12 h light:12 h dark cycle. The animals had free access to standard pellet diet, tap water was given ad libitum. The protocol for this study was approved by

the ethics committee for Animal Research of the Federal Rural University of Rio de Janeiro (COMEP–UFRRJ) under number 002/2009. Control and experimental groups consisted of 6 animals each. The investigated compounds were administered orally (p.o.) as the suspension in 5% ethyl acetate (vehicle) in constant volume of 5 mL/kg.

4.2.2. Statistical analysis

All experimental groups were composed of 6 animals. The results are presented as the mean ± SD in the rota-rod and open field tests, and percentage increase over the baseline or area under the curve (AUC) in the tail-flick test. Statistical significance between groups was performed by the application of one way analyses of variance (ANOVA) followed by Bonferroni's test. $P < 0.05$ was considered as statistically significant. The estimated ED_{50} value (the dose producing 50% of the maximal effect) for the antinociceptive action was obtained by fitting the data points representing the antinociceptive effect demonstrated in acetic acid-induced writhing method by nonlinear regression (sigmoidal dose response) using the GraphPad Prism software version 5.0 (San Diego, CA, USA). The estimated LD_{50} was obtained by fitting the data points representing the percentage of deaths with increasing doses of the compounds up to 2000 mg/kg calculated by nonlinear regression method using the Graph Pad Prism software version 5.0 (San Diego, CA, USA).

4.2.3. Antinociceptive evaluations

4.2.3.1. Acetic acid-induced abdominal writhing. Mice were used as described previously.[24] In brief, the total number of writhes after the ip administration of 1.2% (v/v) acetic acid was recorded over a period of 30 min, starting immediately after acetic acid injection. The pattern of abdominal writhes is the appearance of strong abdominal contractions, stretching the body of the animal, followed by elongation of hind limbs and abdomen contact with the floor of the counting chamber.

4.2.3.2. Tail-flick test. The test was performed as previously described.[25] The mice were kept in an acrylic tube and then placed on equipment to perform tail-flick test. A light beam is focused to approximately 4 cm from the tip of the tail and the tail withdrawal latency is automatically registered. The light intensity was adjusted for baseline values between 4 and 6 s; this intensity was not changed and the animals that had baseline values outside these limits were excluded from the experiment. Measures of latency time were made at intervals of 20 min between each one. The first two measures were made before drug administration. The average of these measures is called 'baseline'. After drug administration six measures of the latency time was performed. Anti-nociception was quantified as either the (IBL) percentage increase over the baseline at each measurement time, or the area under the curve (AUC) of responses from 20 to 120 min after drug administration, calculated according to the following formula based on the trapezoid rule: [AUC = 20× IBL [(20 min) + (40 min) + . . . + (120 min)/2].

4.2.4. Locomotor activity

4.2.4.1. Rota-rod performance test. The rota-rod performance test is an established method for evaluating motor impairment and ataxia.[26] The day before the test the animals were trained twice to maintain the equilibrium for 5 min on a roller apparatus ('Rotarod for mice', U. Basile, Italy). The speed selector was set to 10 rev/min. Twenty-four hours later, mice were treated orally with new hybrids (10 mg/kg, p.o.) and vehicle; and 60 min after administration were placed on the roller for 5 min. Neurological deficit was evaluated by the inability of the animal to remain on the roller for the test period and reported as number of falling of animals.

4.2.4.2. Open-field test. The procedure was similar to the method described by Barros et al.[27] Mice received new hybrids (10 mg/kg, p.o.) and vehicle by oral administration and were placed individually in an observation chamber (60 min after oral administration) whose floor was divided into 50 squares (5 cm × 5 cm). Total numbers of squares by which mouse walked during 5 min were counted. The spontaneous activity was quantified as either number of squares walked within 5 min after compound administration.

4.2.5. In vivo toxicological evaluation of tetrahydropyran derivatives

Acute toxicity test was performed according to the World Health Organization (WHO) guideline[28] and the Organization of Economic Co-operation and Development (OECD) guideline for testing of chemicals.[29] The investigated compounds were administered orally in increasing doses up to 2000 mg/kg. The animal behavior was observed from 5 h after a single administration of the compounds and subsequently monitored daily until the 14th day. Acute toxicity was expressed by the required dose in g/kg body weight to cause death in 50% of animals tested (LD_{50}).

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Supplementary data

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